THE [illegible] OF PROTEIN A [illegible]
[illegible] (*Pennisetum* [illegible]
[illegible] and E. C. Hubbard [illegible]

By

JAMES BYRUM BARNETT

[illegible]ATION PRESENTED TO THE GRADUATE [illegible]
THE UNIVERSITY OF FLORIDA IN PARTIA[illegible]
[illegible] OF THE REQUIREMENTS FOR THE [illegible]
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1975

To

[illegible], our children, [illegible],

[illegible] and my wife's mother, [illegible]

[illegible] sacrificed and willingly [illegible]

[illegible] the completion of this [illegible]

[illegible]

[illegible] from the members of my [illegible] has contributed not only to th[illegible] [illegible] my total education. Dr. Rex L. Smith, [illegible] chairman, has always been available for discu[illegible] [illegible]nling aid. Dr. James Soule gave me a better [illegible]ling of fruit crops, Dr. V. E. Green, Jr., impart[illegible] [illegible]e some knowledge and appreciation of tropical agricul[illegible] and agronomy. Dr. R. C. Robbins' assistance and gu[illegible] in the analytical phase of this study was invaluable. [illegible] R. C. Littel's critique of the statistical analysis ga[illegible] [illegible] better understanding of that discipline.

I consider each a close personal friend and am honored [illegible]hey served on this committee.

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[illegible]

By

[illegible] Bynum [illegible]

June, 1975

[illegible] Rex L Smith
[illegible] Department Agronomy

[illegible] diallel cross was made using inbred [illegible] of [illegible] selected on the basis of variation in prote[illegible] [illegible] content The F_1 generation was planted in [illegible] [illegible] block design at two locations with two replic[illegible] at each location Each replication contained th[illegible] inbred lines produced by selfing and the 30 progeny [illegible]uced by crossing the five lines in all possible combin[illegible]

Total protein (N x 6 25) was determined by rapid micr[illegible] technique Statistical analysis indicated protei[illegible] [illegible] is influenced by the presence of dominant [illegible] for lower protein with evidence of maternal [illegible] influenced by environment Regression [illegible] indicated no linear relationship between total [illegible] amino acids and total protein

[illegible]ysis of lysine inheritance was based upon a com[illegible] amino acid analysis of samples representing a comple[illegible]

[illegible] thre[illegible] [illegible] made com[illegible] [illegible] ibl[illegible] [illegible]ssion analys[illegible] [illegible]ip exists between lysine a[illegible] [illegible] with a very low correlation coeffi-[illegible] [illegible] correlation between lysine and [illegible] [illegible] correlation exists between lysin[illegible] [illegible] and threonine.

[illegible]iques for protein and a rapid quanti-[illegible] [illegible]s technique proved unreliable when used in [illegible] The cause of this unreliability was undeter-[illegible]d.

INTRODUCTION

[illegible] Protein Problem

[illegible] cereal grains are the major [illegible] human diet while legumes and animal pro[illegible] supplemental sources. Nicol (1971) reports [illegible] contribute 63% of the total dietary [illegible] in West African diets while rice contributes up to [illegible] total dietary protein in the Far East. Estima[illegible] contribution of cereal grains to the dietary [illegible] Latin America is complicated by the fact that in [illegible] is the staple grain while in others corn is [illegible] in the diet. Altschul (1965) estimates [illegible] produced more protein for human consumption [illegible] animal and legume sources combined. The importance [illegible] quality and quantity in cereal grains is secondary [illegible] developed countries where animal products are the major [illegible] of protein.

[illegible] prominence of cereal grains as a major protein [illegible] in human diets and problems which are inherent have [illegible] only begun to be elucidated. Howe et al. (1965) [illegible] that where sufficient cereals to satisfy the caloric

[illegible] rable to animal protein [illegible] analyzed on a whole-grain [illegible] recommended levels in one or more essential amino [illegible] ine, for example, is the primary limiting amino [illegible] all cereal grains. Threonine is second limiting [illegible] in maize where tryptophane and lysine are co-limiting (Table 1)

[illegible] concomitant problem is the loss of protein during [illegible]. The principal source of this loss is through [illegible] where the germ, which is proportionally higher in [illegible] than the endosperm, or the seed coat, including the [illegible] layer, may be removed. Loss of either or both of [illegible] fractions lowers both the quality and the quantity of protein in the finished product.

[illegible] ying degrees of protein malnutrition often results [illegible] ing areas of the world where the average diet con[illegible] cereal grains of low nutritive value supplemented [illegible] ll amounts of animal or legume protein. This form [illegible] trition is debilitating in adults. In pre-school [illegible] whose protein needs are high in relation to caloric [illegible] ents, severe cases of protein malnutrition result [illegible] permanent impairment of mental capacity. The caloric [illegible] irement may be met with a cereal-based diet after wean[illegible] a protein deficiency will result unless sufficient

[illegible]

[illegible] OF ESSENTIAL AMINO [illegible]
[illegible]S IN CEREAL GRAINS
[illegible]RESSED IN g/16g N

[illegible]	[illegible]millet[+]	Sorghum[+]	Wheat[+]	Rice[++]	FAO[+++]
[illegible]	[illegible]	2.5	2.7	3.26	4.2
[illegible]	[illegible]	2.1	2.1	3.22	2.4
[illegible]	[illegible]	4.1	3.3	4.03	2.8
[illegible]	2.5	2.7	2.5	2.2	2.2
[illegible]cine	4.3	4.9	3.6	4.0	4.2
[illegible]phane	2.3	1.1	1.2	1.68	1.4
[illegible]	5.7	4.8	4.5	5.75	4.2
[illegible]	17.4	24.2	6.8	8.53	4.8
[illegible]ylalanine	4.9	4.9	5.7	4.4	2.8
[illegible]sine	---	---	---	1.89	---

[illegible]ce: [+]Burton et al., 1972.

[++]Juliano et al., 1968.

[+++]Suggested FAO requirements for good amino acid balance (Food and Agr. Org., 1957).

[illegible] protein sources or supplies. [illegible] is imperative that good quality pr[illegible] [illegible] cheaply as possible. Improving the pr[illegible] [illegible] quantity in cereal grains appears to be a [illegible] to this problem.

[illegible]ulation pressures and cultural mores also contribute [illegible] in problem. Available arable land is decreasing [illegible] population is increasing and the competition between [illegible] animals for the available cereal grains is becoming [illegible] pronounced. Per capita consumption of animal products [illegible]eases as people become more affluent. This also cre[illegible] a pressure on available grain supplies forcing the [illegible] of both grain and animal products upward. This trend [illegible] both grain and animal products less attainable by the [illegible]y of the population in underdeveloped countries where [illegible]ment is high and wages are low.

Cultural patterns and mores often limit the progress [illegible] might be made in improving protein quality and quan[illegible] [illegible]ocal diets. Improved high protein rices are con[illegible] less palatable in some areas of Asia (Juliano et al., [illegible] High lysine corn has not found acceptance in many [illegible] Latin America. Local taboos and prohibitions often [illegible] potential sources of animal protein. Dietary [illegible]tural patterns are difficult to change and progress [illegible] slow where change is required for improved protein [illegible]

[illegible] in balance from plant sour[illegible] can be [illegible] n of plant foods that compliment one [illegible] essential amino acids. A cereal-legume mixture [illegible] eaten in proper balance will often fulfill [illegible] requirements (Johnson et al., 1968). However [illegible] ns and lack of understanding of nutri[illegible] frequently rule out food selection as [illegible] approach to improved diets. Moreover, children [illegible] restricted during their most critical stage of develop[illegible] to what is available in the home.

Improved protein balance can also be accomplished by [illegible] supplemental amino acids in processed foods. The diffi[illegible] of this approach is implementation of the program [illegible] people in greatest need of such a program normally con[illegible] very little commercially processed food. It is either [illegible] available or too expensive.

Another alternative, which also has limitations, is [illegible] improvement of protein quality and quantity in cereal [illegible]. Several approaches have been suggested by Mertz [illegible] most of which are based upon selection and breeding. [illegible] cautions in a program of quality improvement are to [illegible] in the proper balance between essential and non-[illegible] amino acids in the grain and (2) avoid selection [illegible] nutrient without regard to total composition [illegible] grain (Munck, 1964). A third precaution concerning [illegible] lity and acceptability would also be appropriate in

[illegible] with high protein rice and [illegible]

[illegible]

[illegible] Solutions to the Problem

[illegible] made over the last half century [illegible] increased production with little [illegible] nutritional quality. Kamra (1971) [illegible] progress to a general lack of awar[illegible] [illegible] requirements of monogastric animals [illegible]ty of simple, quantitative, inexpen[illegible] [illegible]ive mass screening methods to aid in [illegible] [illegible]ding material. A general lack of [illegible] importance of cereal grains and imbalance [illegible]ls in the diets of developing cultures [illegible] attributed to this situation.

[illegible] in the improvement of protein in some cereal [illegible] early in the twentieth centruy. Woodworth et al [illegible] reported on the results of 50 generations of selection [illegible] in and oil content in corn and shortly afterward [illegible] 1949, 1951) published on the inheritance of protein [illegible]. These studies indicate crude protein could be [illegible] in corn but no serious work was begun since the [illegible] was largely in the form of nutritionally poor zein. [illegible] of the opaque-2 and the floury-2 genes which [illegible]ntly alter the amino acid patterns in maize [illegible] Nitos, 1964, 1965) created renewed interest in [illegible] in cereal grains

[illegible] quality and the quantity [illegible] [illegible] received increasing attention [illegible] the [illegible] International Rice Research Institute [illegible] variability of protein content [illegible] Asia (Juliano et al., 1964) and later [illegible] high protein rice varieties (Juliano [illegible] Nebraska Agricultural Experiment Station [illegible] screened 16,000 selections of wheat for [illegible] protein and lysine differences. The data indicate [illegible] increase in protein content of wheat can be [illegible] without sacrificing yield (Johnson et al., [illegible]). Lower yield in high protein lines has been the major [illegible] in the introduction of high protein commercial [illegible]. A sorghum program was begun at Purdue University [illegible] investigate protein quality and quantity in that crop. [illegible] result of this program was the discovery of a high [illegible] mutant gene (Singh and Axtell, 1973). A broad-scaled [illegible] designed to upgrade protein quality and quantity [illegible] cereal grains was also developed in India (Swaminathan [illegible] 1971).

The inferior quality of cereal proteins is attribut- [illegible] the ratio among the protein fractions in the grain [illegible] low in lysine content constitute the major per- [illegible] (40-60%) of seed proteins in most cereals, with [illegible] of intermediate lysine content constituting most [illegible] remainder. The smallest protein fraction in cereals

[illegible] (Nelson, 1969). These di[illegible] among various protein fr[illegible] possibility of improvement through [illegible] would suppress the synthesis of protein [illegible] in the desired amino acids with a compensat[illegible]is of other fractions with higher levels of the [illegible] acids.

[illegible]ynthesis of amino acids is regulated to correspond closely to demand for incorporation into proteins by the plant, therefore, cereal grains contain very low quantities [illegible] free amino acids (Nelson, 1969). An induced or spontaneous mutation causing a loss of sensitivity in control of [illegible]o acid synthesis could cause an over-synthesis with a [illegible]lting increase in free amino acids. No mutation of [illegible] type has been identified to date (Nelson, 1969).

The more promising types of mutations are those which [illegible] gross change in the structural components of the [illegible] or seed structures. The high lysine mutants in [illegible] of this gross structural change type. Theoreti[illegible] e-2, the high lysine gene, inhibits the synthe[illegible] the prolamine fraction of the protein, in maize. [illegible] of mutations in which one amino acid is sub[illegible] for another in protein synthesis have not been [illegible] in changing protein quality and quantity.

It became increasingly apparent as the components ... [illegible] ... presented that significant changes in ... [illegible] ... tion of cereal protein could result ... mutations. More specifically these changes ... mutations which reduce synthesis of the ... prolamine fraction and increase synthesis ... in fractions (Johnson et al., 1968, Munck, ...) and Bates (1964, 1965) found a significant ... in corn, the prolamine fraction of corn protein, ... homozygous for either the opaque-2 or ... gene with a corresponding increase in lysine. ... (1965) found the same effect when the opaque-2 ... incorporated into various races of corn. More ... and Chang (1974) studied the effect of ... protein and lysine content in corn by incorporating a gene conditioning multiple aleurone cell layers, ... are high in lysine.

Significant improvement in protein quality or quantity in cereal grains can be accomplished through selection ... and by screening mutations, either spontaneous ... Manipulation of major genes offers the most ... and the most rapid method of increasing protein ... or quantity but generally major genes are not ... Selection and breeding hold the most promise ... improvement when sufficient variability is ... The researcher can attempt to induce mutations

[illegible] improvement [illegible] [illegible] matic in changing [illegible] quantity [illegible] be concentrated [illegible] [illegible] are the most [illegible] also [illegible] predictable [illegible] the genetic procedures.

[illegible]s which may influence the total protein or the [illegible] certain amino acids in the grain should be con[illegible] as well as the form in which the grain is consumed. [illegible]ences in structural components of the seed have a [illegible] influence on their ability to transmit genetically [illegible]essed protein and amino acid potentials. Milling pro[illegible]res and cooking methods may remove portions of the seed [illegible]h are highest in good quality protein. Cultural prac[illegible]s may also significantly alter both the quality and the [illegible]ntity of protein in the endosperm (Sawhney and Naik, [illegible]0).

Proteins in the germ are much superior in protein [illegible]lity to those of the endosperm (Nelson, 1969), therefore, [illegible]reasing the relative size of the embryo for quality and [illegible]tity of protein in the diet would be of importance where [illegible] whole seed is consumed. No studies have been made to [illegible]re the possibilities of significantly changing the [illegible] acid composition of the embryo but it has been noted [illegible] both the opaque-2 and the floury-2 genes of maize [illegible]se embryo size when incorporated into new lines

[illegible] grain. [illegible]

[illegible] significantly [illegible] the thickness [illegible] [illegible] increase both the quality [illegible] quantity of protein in the grain. The improved protein [illegible] the amylose extender gene in maize is believed [illegible] the increased thickness of the aleurone layer [illegible].

[illegible]erior quality of cereal proteins may be attributed [illegible] small fraction of globulins which are high in lysine [illegible] the large fraction of prolamines of low lysine content [illegible]ulins are abundant in the aleurone layer and the embryo [illegible] prolamines are found in the endosperm. Any factor [illegible] significantly alters the ratio of these protein frac- [illegible] will have significant effects on the protein quality [illegible]tity of the grain

[illegible]spect of the problem unrelated to the breeding [illegible] is the lack of a simple, quantitative, non- [illegible]ve method of analysis for total protein and amino [illegible]

[illegible]nding techniques have been developed which [illegible] protein in some cereal grains. Udy (1971) developed [illegible] technique originally for wheat and later expanded it to

[illegible] (1967) [illegible]
[illegible]
[illegible] for the [illegible]
[illegible] grains. The method [illegible]
[illegible] to isolate the high lysine [illegible]
[illegible] (1969) described a method of [illegible]
[illegible] rice grains for protein content [illegible]
[illegible]ding technique. Johnson and Craney (1971) reported
[illegible] biuret method for estimation of protein in grains.
[illegible] and Mooney (1970) found a high correlation among
[illegible] from dye-binding, biuret, and micro-Kjeldahl
[illegible]es. Concon and Soltess (1973) described a rapid
[illegible]o-Kjeldahl process in which digestion is completed
[illegible]in 10 minutes.

The major problem confronted in the search for a rapid [illegible]tative analytical technique for lysine or total amino [illegible] is hydrolysis. Standard methods require special [illegible]nt and a great deal of time. Falter and Kohler (1969) [illegible] a survey hydrolysis procedure in which up to 300 [illegible] per week may be processed, but even this method is [illegible] to meet the requirements of a breeding program in [illegible]sands of segregating genotypes must be analyzed. [illegible]itative analytical methods for lysine include [illegible]graphic (Zschcile and Brannaman, 1972), [illegible] (Concon, 1972. Ghosh and Bose, 1973), and thin-[illegible]matographic techniques (Heathcote and Haworth,

[illegible]

[illegible]millet [illegible] (Pennisetum typhoides (Burm.) [illegible] Hubbard) is a robust annual bunchgrass grown on 20 [illegible]ion hectares as a grain crop for human consumption ([illegible]ton and Powell, 1968; Burton et al., 1972). It is [illegible]dapted to the tropics and will grow and mature seed [illegible] is too infertile and too dry for other grain crops [illegible]ity to produce in the dry, infertile areas of the [illegible] has made it a major food source. Good nutritional [illegible] of the grain justify the research in production [illegible]ovement of this crop presently being advocated ([illegible] 1969).

[illegible]rl millet has a protein content ranging from 8.8% [illegible] with lysine varying from 1.9% to 3.4% of the pro[illegible] (Burton et al., 1972, Wallace, unpublished data) [illegible] are in agreement with those of Swaminathan et al., [illegible] 8 to 20% protein, with lysine ranging from [illegible] the protein, and tryptophan ranging from [illegible] of the protein. Losses occur during milling, [illegible]ately 85% of the proteins in the whole grain [illegible]rmally remain after processing (Dewit, 1969).

Millet [illegible] content and balance of essential [illegible] is equal to or better than most other cereals [illegible] is higher than rice, wheat, or maize in fat and [illegible] (Table 2).

[illegible] yield potential has not been established [illegible] there is evidence to suggest pearlmillet yields may compare favorably with corn and sorghum where good management including use of adopted varieties, irrigation, and fertilizers was practiced (Burton and Powell, 1968; Burton et al., 1972).

Purposes of this study were (1) to determine the [illegible]tibility of protein and lysine, the primary limiting [illegible], in the grain of pearlmillet and (2) to investigate [illegible] qualities and adaptability of pearlmillet of [illegible] [illegible]titative methods of analysis reported in the [illegible] for protein and lysine

TABLE 2

OIL AND MINERAL COMPOSITION OF
SOME CEREAL GRAINS

[illegible]	Oil %	Ca mg	[illegible]
[illegible]	4.5	46	[illegible]
[illegible]	1.8	46	[illegible]
[illegible]	3.9	22	[illegible]
[illegible]—brown	1.9	32	[illegible]
[illegible]	3.3	28	[illegible]

[illegible] Composition of Foods. USDA Agriculture Handbook No. 8

MATERIALS AND METHODS

The Diallel Cross

[illegible] was made during the summer of [illegible] [illegible] received from Tifton, Georgia. [illegible] the basis of variation in protein and [illegible] [illegible] by Wallace and Block (unpublished) [illegible] [illegible] 3.

TABLE 3

PROTEIN AND LYSINE CONTENT OF INBRED LINES USED IN THE DIALLEL CROSS

[illegible]	Protein %	Lysine (as % protein)
[illegible]	11.24	2.[illegible]
[illegible]	15.31	2.58
[illegible]	10.12	2.53
[illegible]	10.31	3.70
[illegible]	16.12	2.15
[illegible]	19.12	2.00

Progeny from the diallel cross were planted in a split plot design over two locations with two replications at each location. Replications contained the six inbred lines produced by selfing the parental lines and the 30 F_1s (including reciprocals) derived by crossing the six lines in all possible combinations.

Location I was planted on the Agronomy Farm at the University of Florida, Gainesville. Each plot consisted of three rows 5.5 m in length, with 1 m between rows. Plants were spaced approximately 0.5 m apart in the rows. One application of 403 kg/ha of 10-10-10 fertilizer was broadcast before planting. Heads from the center row of each plot were randomly selected and bagged with a kraft bag and an aluminum screen protector bag to minimize bird damage. Heads remained on the plants until they were fully matured.

Location II was planted the same season (summer, 1974) on the Morikami Farm, University of Florida Experiment Station, Delray Beach, Florida. Plots were bedded due to the high water table in this area and covered with black plastic to minimize weed competition. The beds were approximately 30 cm high and 1.5 m wide. The plots were fertilized with 403 kg/ha of 10-10-10 fertilizer broadcast prior to bedding. Each plot consisted of two rows 90 cm apart and 5.5 m long placed upon the bed. Plants were spaced approximately 0.5 m apart in the rows. Bedding and covering the beds with plastic minimized interplot competition. Heads from both

[illegible] randomly selected and [illegible] aluminum screen prot[illegible] plants until they were full[illegible]

[illegible] were harvested and three h[illegible] from each plot for use in the quant[illegible] [illegible]in and lysine. Line I-18 and its pr[illegible] [illegible] to poor germination and poor seed s[illegible] [illegible] plots.

Analytical Procedures

[illegible] measures of seed from each of the three heads [illegible] bulked for estimation of the plot mean for [illegible] lysine. Samples were ground in a Norris hammer [illegible] a [illegible] mm screen and oven dried for 24 hours at [illegible] before analysis. Samples used in the analysis for [illegible] and the dye-binding capacity (DBC) technique for [illegible]on of protein were further ground in a mortar and [illegible]sing approximately 5 ml acetone as a wetting agent

[illegible]alysis

[illegible]in (N x 6.25) was determined by the rapid micro-[illegible] procedure described by Concon and Soltess (1973) [illegible]y 2.3 g of K_2SO_4/MgO mixture and 2.3 ml H_2SO_4 [illegible] to a ground sample of 50 mg. The mixture was [illegible]til frothing occurred then approximately 1 ml 30% [illegible] added. Heating at a lower temperature, approximately

[illegible] 1965) [illegible] verified by correlating results [illegible] with those from the [illegible] Data generated by the micro [illegible] used to determine the accuracy of [illegible] for protein estimation in pearl [illegible]

[illegible]

[illegible] samples of 100 g each were hydrolyzed [illegible] HCl in a nitrogen-saturated atmosphere at 40° C [illegible]. The solute was brought to dryness in a rotary [illegible] at 40° C and added with 10 ml 0.01N HCl. A 1:10 [illegible] tion was used for the complete amino acid determi[illegible]

[illegible] samples representing a complete set of crosses [illegible] lication, including inbreds but not reciprocals, [illegible] on a Jeol Model JLC-6AH Amino Acid Analyzer.

[illegible]

[illegible] was estimated as grams of lysine/16 g N using [illegible] lysine determination technique described by [illegible] 72). A protein extraction was made from a 500 mg [illegible] ground seed using 1.5 ml 70% ethanol followed by [illegible] NaOH. Nitrogen content of the extract was [illegible] by rapid micro-Kjeldahl procedures (Concon and

[illegible] absorbance [illegible] in the literature. [illegible] vity at 360 nm to distingu[illegible] ces were visually apparent [illegible] this procedure was verified by correlating [illegible] data on 15 samples analyzed with the amino [illegible]

Statistical Analysis

[illegible]rotein data were analyzed using Griffing's (1954) [illegible]hod 3 (in which all F_1s are used), model I (all effects [illegible]t error are considered fixed) for estimates of general [illegible]ning ability (GCA) and specific combining ability [illegible] protein inheritance. Method 3, model II, in which [illegible]cts are considered random variables, was used to [illegible] heritability with the formula for the genetic [illegible] estimate (σ^2_G) modified to include the reciprocal [illegible]timate (σ^2_r). Estimates of heritability (h^2) in [illegible] were calculated.

[illegible] same data were analyzed by the Hayman (1954) [illegible] of diallel cross analysis for estimation of genetic [illegible]. Gene action parameters estimated by this method [illegible]itive, dominance, and maternal effects;

RESULTS

Protein Inheritance

A highly significant difference was found between crosses. All other parameters were non-significant (Table 7). Location differences were non-significant but observation of mean values of crosses compared across locations (Table 4) suggests the environmental effect upon crosses was variable in both the positive and the negative directions masking possible location effects.

Table 5 and 6 contain the means of each cross (by location) arranged with the inbred parents forming the diagonal. In this format reciprocals are also located diagonally from one another. Reciprocal differences in some lines are apparent.

Combining Abilities

Data at both locations were subjected to analysis of variance for combining ability (Tables 8 and 9). Estimates of GCA and SCA differed with the reciprocals showing no significant difference between locations. Parental reciprocal estimates ($\hat{s}_r^2$) (Tables 10 and 11) indicate the major reciprocal differences of approximately the same magnitude

Parents		Gainesville		D[illegible]	
Female	Male	Rep. 1 (%)	Rep. 2 (%)	Rep (%	[illegible]
I-23 (selfed)		16.71	16.13	[illegible]	[illegible]
I-23	T-27	13 39	13 92	[illegible]	[illegible]
I-23	T-140	14.38	15.80	[illegible]	[illegible]
I-23	T-4	12.44	18.46	10.[illegible]	[illegible]
I-23	T-98	14.77	13 22	13 61	[illegible]
T-27 (selfed)		19.63	19 70	23 48+	[illegible]
T- 7	I-23	20.99	20.60	21 18	21 83
T-27	T-140	14.96	14.38	14 58	[illegible]
T-27	T-4	14 96	15.35	10.11	[illegible]
T-27	T-98	17 25	17.88	12.63	[illegible]
T-140 (selfed)		23 32	22.93	21.54	[illegible]
T 140	I-23	13 99	13 [illegible]	[illegible]	[illegible]

[illegible]	[illegible]	[illegible]	Exp. 2	[illegible]	[illegible]
[illegible]	T-27	15.94	[illegible]	[illegible]	[illegible]
[illegible]	T-4	17.48	[illegible]	[illegible]	[illegible]
I-140	T-98	14.96	[illegible]	16.91	[illegible]
T-4 (selfed)		16.71	17.10	19.82	[illegible]
T-4	I-23	17.53	13.21	14.77	[illegible]
T-4	T-27	19.05	18.08	12.63	[illegible]
T-4	T-140	17.36	17.38	16.33	[illegible]
T-4	T-98	17.49	17.49	14.27	[illegible]
T-98 (selfed)		17.63	17.68	19.44	[illegible]
T-98	I-23	15.68	16.48	16.52	[illegible]
T-98	T-27	14.38	15.74	[illegible]	[illegible]
T-98	T-140	17.43	18.27	[illegible]	[illegible]
T-98	T-4	14.97	15.55	[illegible]	[illegible]

+Missing data estimated by [illegible]

TABLE 5

MEAN PR[illegible] CONTENT OF ALL [illegible]
OF A DIALLEL CROSS, GAINESVI[illegible]

[illegible]	Male parents				[illegible]
[illegible]	1-23	T-27	T-140	T-4	[illegible]
			(%)		
[illegible]	16.42	13.92	15.09	15.45	[illegible]
[illegible]	20.79	19.66	14.67	15.15	17.[illegible]6
[illegible]	13.79	16.03	23.12	17.68	14.86
[illegible]	15.40	1[illegible].56	17.37	16.90	17.49
[illegible]	16.08	15.06	17.98	15.26	17.68
[illegible]					

TABLE 6

[illegible] CONTENT OF ALL PROGENY [illegible] CROSS, DELRAY BEACH

[illegible]	Male parents				
[illegible]	[illegible]	T-27	T-140	I-4	[illegible]-98
			(%)		
[illegible]	14.96	14.77	15.94	13.02	13.75
[illegible]	21.51	23.48+	14.79	10.11	11.37
[illegible]	17.22	12.70	21.46	[illegible]	17.40
[illegible]	16.32	14.34	17.01	19.43	15.76
[illegible]	15.55	11.07	14.15	16.52	19.05

[illegible] value estimated by Yates method (Cochran and Cox, [illegible]

TABLE 7

ANALYSIS OF VARIANCE FOR PROTEIN OF PROGENY RESULTING FROM A DIALLEL CROSS WITH FIVE INBRED PARENTS

Source	df	MS
Locations	1	12.945
Error (a)	2	1.164
Crosses	24	25.123**
Locations x Crosses	24	0.572
Error (b)	48	4.49

**Significant at .01.

TABLE 8

[illegible] ANALYSIS FOR COMBINING ABILITY FOR PROTEIN, GAINESVILLE

[illegible]	df	Mean squares
[illegible]		
[illegible]	4	2.751
[illegible]	5	2.106
[illegible]	10	4.359
[illegible]	48	2.[illegible]74

TABLE 9

[illegible] ANALYSIS FOR COMBINING ABILITY FOR PROTEIN, DELRAY BEACH

[illegible]	df	Mean square
[illegible] ability		
[illegible]	4	7.991*
[illegible]ific	5	9.875**
[illegible]	10	4.765*
[illegible]	48	2.24

*[illegible]ificant at .05

**[illegible]ificant at .01

[illegible] 10

[illegible] SCA, AND RECIPROCAL VA[illegible] [illegible] EACH PAR[illegible]NT, AND ENVIRONM[illegible] [illegible] FOR [illegible]OTLIN, GAINESV[illegible]

[illegible]	[illegible]	$\hat{\sigma}^2_s$	$\hat{\sigma}^2_r$	[illegible]
[illegible]	[illegible]	0 0025	3 4238	0 [illegible]
[illegible]	[illegible]	0.5983	4 5627	0.1269
[illegible]	-0.4476	1.8200	0.0487	0 1269
[illegible]	-0.1614	0.9769	0.3773	0.1269
[illegible]	-0 4953	-0 5026	1.0450	0 [illegible]

TABLE [illegible]

[illegible]S OF GCA, SCA, AND RECIPROCAL VA[illegible] [illegible]TED WITH EACH PARENT, AND ENVIRONMENTAL [illegible]ANCE MEANS FOR PROTEIN, DELRAY BEACH

[illegible]	σ^2_g	σ^2_s	σ^2_r	[illegible]
[illegible]	1 4735	3 9958	4.0854	0 [illegible]
[illegible]	1 9195	4 0026	4 6339	0 [illegible]
[illegible]140	0.4737	0.5017	0.4172	0 [illegible]
[illegible]4	-0.3991	0.5833	1.4831	0 [illegible]
[illegible]98	0.0937	1.4675	0 1915	0 [illegible]

[illegible] crosses at both [illegible] had little effect [illegible] lines I-23 and I-27. [illegible] an influence on progeny from [illegible], however. Differences in combining [illegible] locations are an indication of the eff[illegible] [illegible] on protein content.

[illegible] GCA of each of the five inbred lines in this study [illegible] significant at Gainesville. Estimates associated [illegible] each parent show line I-23 superior to all other lines [illegible] location, this being evidenced by the consistency [illegible] progeny in approaching the parental value in protein [illegible] (Table 5).

The GCA variance estimate ($\hat{\sigma}_g^2$) associated with each parent indicates lines T-27 and I-23 are superior to all other lines tested at that location (Table 11). The most striking difference in the parental GCA variance estimates between locations is that line T-4 has a negative value [illegible] four lines have negative values at the Gainesville [illegible]ion.

Analysis of SCA shows non-significant effect at Gaines[illegible] (Table 8) and a highly significant effect at Delray [illegible] Tables 8 and 9). Parental estimates for SCA ($\hat{\sigma}_s^2$) [illegible] line T-140 to be superior to other lines at Gainesville [illegible] 8) and lines T-27 and I-23 superior at Delray Beach [illegible] 10 and 11).

... were estimated assuming ... twice the GCA variance (σ^2_g) ... genetic variance was estimated by the ...

$$\hat{\sigma}^2_G = 2\hat{\sigma}^2_g + \hat{\sigma}^2_s + \hat{\sigma}^2_r$$

where

$\hat{\sigma}^2_G$ = total genetic variance,

$\hat{\sigma}^2_g$ = estimate of GCA

$\hat{\sigma}^2_s$ = estimate of SCA, and

$\hat{\sigma}^2_r$ = estimate of reciprocal effects

The phenotypic variance ($\hat{\sigma}^2_P$) was estimated by the ...

$$\hat{\sigma}^2_P = \hat{\sigma}^2_G + \hat{\sigma}^2_e$$

... error.

... lity is then estimated by the formula

$$h^2 = \frac{2\hat{\sigma}^2_g}{\hat{\sigma}^2_P}$$

... narrow sense and

[illegible]

[illegible] itability estimate [illegible] to the negative v[illegible] (Table 12). Broad-sense heritabilit[illegible] xtremely variable between locations with a value of [illegible] at Gainesville and 0 54 at Delray Beach

[illegible]ic Parameters

The Wayman (1954) method of diallel analysis indi[illegible] dominance for protein content. The negative values [illegible]an squares were interpreted as indications of domi[illegible] for lower protein content (Table 13). The consis[illegible] with which the progeny deviated in the negative [illegible] on from the mid-parent value (the mean of the [illegible]) for protein content confirms this interpretation [illegible]nalysis of data also indicates no significant [illegible] difference among parental lines The Yates (1947) [illegible] of interpretation of data reveals a significant [illegible] effect which is evidenced by significant re[illegible] differences involving some lines

[illegible]

[illegible] OF VARIANCE
[illegible] RECIPROCALS OF [illegible]

Source of variation	df	Gainesville MS	Variance	MS	[illegible]
GCA	4	1.751	-0.037	7.991	-0.126
SCA	5	2.306	-0.463	9.675	[illegible]
Reciprocal	10	4.359	0.554	4.765	[illegible]
Error	46	5.06		5.06	

TABLE 1[illegible]

[illegible]ANCE FOR GEN[illegible]
[illegible]IN INHERITANC[illegible]

[illegible]	df	[illegible]
[illegible]	4	[illegible]
[illegible]	10	-883.3[illegible]
[illegible] EFFECT	4	17.1[illegible]
[illegible]	6	[illegible]

[illegible] Inherit[illegible]

[illegible] tance for lysine [illegible] on the amino acid analy[illegible] or the rapid analytical technique [illegible]). There was no replication, hence [illegible] inferences cannot be made. Hypotheses, [illegible] mulated for verification in further work. [illegible] analysis of the data (Table 14) indicates [illegible] tionship exists between lysine and total [illegible]l amino acids but with a very low correlation [illegible]icient. A negative correlation (r = -0.44) exists between [illegible] protein content with a strong positive correla- [illegible] lysine and the sum of methionine and threonine [illegible]. A linear relationship was not indicated between [illegible]al amino acids and total protein although the [illegible] indicate a slight negative correlation may exist.

Quantitative Analytical Methods

[illegible] method for screening samples for protein [illegible] reported by Kaul *et al.* (1970) was unreliable [illegible] pearlmillet (Table 17). The rapid, quanti- [illegible]al technique for lysine reported by Concon [illegible] proved unreliable (Table 16). The rapid micro- [illegible] technique reported by Concon and Soltess (1973) [illegible] accurate and consistent and correlated well [illegible]) with the macro-Kjeldahl results (Table 18).

[illegible]	Lys	His	Thr	Val	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T-23 selfed	[illegible]	1.47	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
T-23 x T-27	1.66	1.79	2.99	[illegible]	[illegible]					[illegible]
T-23 x T-140	1.14	[illegible]	1.89	2.79	1.17	2.18	5.26	[illegible]	[illegible]	[illegible]
T-23 x T-4	1.56	2.05	3.83	6.42	6.64	0.55	4.96	[illegible]	[illegible]	[illegible]
T-23 x T-98	2.27	1.66	2.77	3.47	1.52	2.95	6.85	[illegible]	[illegible]	[illegible]
T-27 selfed	1.04	1.11	0.23	3.80		2.59	6.47	[illegible]	[illegible]	[illegible]
T-27 x T-140	0.68		1.07	1.54	0.92	1.15	2.79	0.97	[illegible]	14.38
T-27 x T-4	1.65	1.33	2.55	3.63	0.87	2.91	6.72	2.06	[illegible]	15.35
T-27 x T-98	1.78	2.05	3.62	5.88	1.14	4.61	[illegible]	[illegible]	5.55	17.88
T-140 selfed	0.79	0.69	1.30	1.92	0.79	1.55	[illegible]	[illegible]	[illegible]	22.93
T-140 x T-4	1.21	1.25	2.39	3.64	1.13	2.90	[illegible]	[illegible]	[illegible]	[illegible]
T-140 x T-98	1.79	1.73	3.80	5.07	1.42	4.37	10.51	[illegible]		[illegible]
T-4 selfed	1.53	1.50	2.94	5.01	0.78	3.92	9.33	[illegible]		[illegible]
T-4 x T-98	0.73	0.73	1.41	2.20		1.81	4.29	[illegible]	[illegible]	[illegible]
T-98 selfed	1.62	1.56	3.38	4.83	0.53	3.90	9.11	[illegible]		[illegible]

TABLE 15

LYSINE CONTENT OF PROGENY
FROM A DIALLEL CROSS

	Male parents				
	[illegible]	1 27	T-140	T-4	[illegible]
[illegible]	[illegible]	1.64	1.14	1.56	[illegible]
[illegible]		1.04	0.68	1.24	1.78
[illegible]			0.79	1.21	1.79
[illegible]				1.35	0.73
[illegible]					1.62

Table [illegible]

[illegible] TECHNIQUE TO [illegible] THE AMINO ACID ANALYZER

[illegible]	(g/100g sample) [illegible]	Extracted*	% Extracted	[illegible] (grams/[illegible]) Amino acid analyzer	[illegible] Concon method
[illegible]	[illegible]	1.3[illegible]	48	1.43	2.63
[illegible]	[illegible]	0.83	28	1.32	1.32
[illegible]	[illegible]	1.13	44	1.13	[illegible].41
[illegible]	[illegible]	2.48	84	1.56	3.74
[illegible]	[illegible]11	1.31	62	2.27	3.34
[illegible]	2.08	1.92	92	0.75	1.33
[illegible]	2.55	1.79	70	1.16	1.94
[illegible]	3.17	1.04	33	1.61	1.45
[illegible]	3.67	1.36	37	0.76	1.08
[illegible]	2.80	2.05	72	1.20	2.13
[illegible]	2.39	2.00	84	1.79	1.36
[illegible]	2.74	1.35	49	1.32	1.45
[illegible]	2.80	0.87	31	0.73	1.57
[illegible]	[illegible].82	1.31	46	1.63	[illegible].00

*[illegible] as described by Concon (1972)

TABLE 17

[illegible] DBC AND MICRO-[illegible]
[illegible]IN, REPLICATION I, [illegible]

[illegible]	DBC values	[illegible]
	Absorbance	[illegible]
[illegible]	.29	[illegible]
[illegible]	.28	[illegible]
[illegible]	.27	[illegible]
[illegible]	.29	[illegible]
[illegible]	.26	[illegible]
[illegible]	.31	19.63
[illegible]	.32	20.99
[illegible]	.32	14.96
[illegible]	.30	14.96
[illegible]	.26	17.25
[illegible]	.31	23.32
140 x [illegible]	.31	13.99
x [illegible]	.26	15.94
x T-4	.30	17.88
x T-98	.26	14.77
[illegible] selfed	.29	16.71
[illegible] I-23	.30	17.59
x I-140	.29	17.36
T-98	.29	17.49
[illegible]lfed	.31	17.64
[illegible] I-23	.30	15.68
[illegible] T-27	.32	14.38
x I-140	.29	17.49
x I-4	.31	14.97

Table 18

[illegible] BETWEEN MICRO- AND MAC[illegible] PROTEIN, REPLICATION II, [illegible]

[illegible]	Macro-Kjeldahl	[illegible]
	% Protein	% Protein
[illegible]	16.12	[illegible]
[illegible]	14.15	[illegible]
[illegible]	15.47	[illegible]
[illegible]	18.84	[illegible]
[illegible]-98	13.59	13.21
[illegible]	19.53	19.70
[illegible]-23	19.78	20.60
[illegible]-140	14.25	14.38
[illegible] 1-4	15.56	15.35
[illegible] 1-98	17.91	17.88
[illegible] elfed	22.78	22.93
[illegible] x 1-23	13.31	13.69
[illegible] 1-27	16.50	16.13
x T-4	17.62	17.49
T-98	14.91	14.96
[illegible] lfed	17.81	17.10
[illegible] 1-23	13.31	13.21
[illegible] 27	18.00	18.09
[illegible]	17.42	17.38
[illegible]	17.44	17.49
[illegible]	18.38	17.18
[illegible]	16.22	16.48
[illegible]27	15.09	15.74
[illegible]-140	17.72	18.[illegible]7
[illegible]	15.00	15.55

r complicated by evidence of maternal influence
..., 1969) and divergence in modes of inheritance in
... the same character may follow different patterns o
... tance in various lines within the same species
... t, 1970)

Another force influencing protein content is the
... r nment. Cultural practices such as the rate and time
... rtilizer application influence protein content of many
... grains (Swaminathan et al., 1971) Increased protein
... rtilizer application increases the prolamine fraction
... the more desirable globulin and albumin
... ulting in protein of a lower biological value
... (1969) reported high protein genetic traits
... associated with a more efficient and complete
... ion of nitrogen from the plant to the grain rather
... differential nitrogen uptake or accumulation by

[illegible] by environmental [illegible] interaction [illegible] sady, 1974 [illegible], (1968), [illegible] et al., 1973), [illegible] millet (Mahadevappa, 1969). [illegible] the phenotypic expression of protein content [illegible] dual plant is a balance between the genetic [illegible] the environmental conditions, and the interac- [illegible] the two.

[illegible] results of this study are further evidence of [illegible] complex nature of protein inheritance. The signifi- [illegible]e of environmental differences due to location appears [illegible] masked by other interactions.

[illegible]nvironmental differences were present, but the effect [illegible]ironment across 25 different genotypes masked this [illegible]. Any statistical analysis of an experiment concern- [illegible] in analysis should, therefore, partition the [illegible] into all possible interactions.

[illegible]fferences between replications in some crosses [illegible] cannot be explained. Seed used in growing the [illegible] was taken from the same head, hence variatio[illegible] [illegible]tributed to possible differences in genotype of [illegible]. This is also verified by the analysis of

[illegible] field, [illegible] an unknown [illegible] within [illegible]

[illegible] of the data [illegible] conclusive but do give some basis [illegible] When individual plots with replication [illegible] counted the Delray Beach location has [illegible] number found in Gainesville. Further [illegible] reveals that in every case either line T-4 [illegible] T-98 or both lines are involved. These same lines [illegible] have divergent reciprocal variances across locations [illegible] 10 and 11). It may be hypothesized that some [illegible] maternal effect on environment interaction is [illegible], when these facts are considered in the light of [illegible] of some maternal effect as indicated by the [illegible] of genetic parameters (Table 13). Environmental [illegible] which caused the higher incidence of replication [illegible] at Delray Beach are unknown.

[illegible] of maternal effects as indicated by the data [illegible] in significant reciprocal variances at both [illegible] Parental variance estimates indicate that lines [illegible] T-27 are responsible for most of the variance due [illegible] effect with either line T-4 contributing some [illegible] Delray Beach or line T-98 at Gainesville. These

[illegible] in pearl [illegible] due to [illegible]

[illegible] effects and [illegible] complicates the breeding of [illegible] protein in the grain. Any program [illegible] should examine not only combining [illegible]. These factors influence not only the [illegible] but also whether a chosen line should be [illegible] or the female parent.

[illegible] have placed a great deal of stress [illegible] estimates for specific genetic traits. [illegible] estimates, however, are valid only where extensive [illegible] has been done with data pooled over genotypes, years [illegible] tions or where specific genotypes and locations are [illegible] (Allard, 1966, Briggs and Knowles, 1967; Robinson, [illegible]

[illegible] ability in estimates of heritability for protein [illegible] not unique to millets. Crook and Casady (1974) [illegible] heritability estimates for this trait in sorghum [illegible] methods of calculations and with the popula[illegible] et al. (1968) found components of additive [illegible] significantly different between locations [illegible] would, in turn, result in significantly [illegible] heritability estimates between locations.

[illegible] for genetic [illegible] [illegible] found [illegible] [illegible] reported by Ahmad et al. (1972) and Maha[illegible] [illegible] similar to those in this study. Pred[illegible] [illegible] action and considerable environ[illegible] [illegible] contribute positively to the denominator of the [illegible] estimate formula. Environmental interactio[illegible] [illegible] could account for the variability in estimates [illegible].

[illegible] in this work indicates that analysis for [illegible] may prove inadequate when working with a [illegible] inherited trait. It should be one of a [illegible] steps leading toward a better und[illegible] [illegible] gene actions involved in the inheritance of [illegible]. Only through such analyses can we discover [illegible] content *per se* is controlled by a major gene [illegible] it is the sum of many genes, each of which [illegible] synthesis of a particular amino acid with [illegible]ions between control mechanisms.

[illegible] limited data on lysine inheritance make detailed [illegible] impossible. Certain correlations can be made, [illegible] and may be useful in the formulation of hypotheses [illegible] study.

[illegible] data indicate, when selection is based solely on pro[illegible] content, higher protein is accompanied by lower value

and threonine

evidence is the negative

and total protein.

absence of a linear relationship between

amino acids and total protein with the very low

ion between lysine and total amino acids indicates

could be made through selective breeding.

three analytical techniques, DBC, the Concon rapid

rapid micro-Kjeldahl, were selected for study

they showed promise of being effective tools in

breeding program designed for protein improve-

for the failure of the dye-binding technique

quantitative lysine technique are unknown,

correlations which could be attributed to

One hypothesis is that part of the lysine

is bound in protein complexes which prevent

or inhibit dye-binding. Singh et al. (1971)

evidence for additive and cumulative gene action

in rice. No other work has been reported on this

CONCLUSIONS

The inheritance of protein in pearlmillet [illegible] influenced [illegible] presence of dominance for low protein with [illegible] [illegible]nal effects which are affected by environmental [illegible]. More data must be collected to estimate these [illegible] and their significance in a breeding program.

The inheritance of lysine may be influenced by the [illegible] of dominance in some lines (Table 15). Lack of [illegible] of replications precluded estimation of environ-[illegible] effects. Regression analyses indicated selection [illegible] total protein may have little effect on total [illegible] amino acids but have a negative effect on [illegible], [illegible], and threonine.

[illegible] quantitative analytical techniques for lysine [illegible] [illegible]table when applied to pearlmillet in this work [illegible] breeding for grain with better protein quality [illegible] [illegible]eded until this deficiency is corrected.

BIOGRAPHICAL SKETCH

[illegible] was born July 30, 1931, [illegible] He attended high school at Memphis Technical [illegible] the University of Tennessee Junior College at [illegible] Tennessee, in 1949

[illegible] the U S Marine Corps at the outbreak of the [illegible] Conflict and was sent to Korea after basic training [illegible] as a radio operator He rose to the rank of [illegible] and in 1953 was recommended for commissioning [illegible] of 2nd lieutenant for demonstrating leadership [illegible] in combat. He was commissioned in March of 1953 [illegible] Martha Hill of Paris, Tennessee, in December of [illegible] year He was released from active duty in 1955 with [illegible] of 1st lieutenant and was honorably discharged in [illegible] with the rank of captain

[illegible] returned to Tennessee upon release from active duty [illegible] attended the University of Tennessee at Knoxville [illegible] Agronomy [illegible] assumed management responsibilities of a family [illegible] in Huntsville, Alabama, upon graduation, and was [illegible] at Redstone Arsenal with the missile industry [illegible] industry in 1962 and accepted employment

[illegible] lic School System as a biology [illegible]

[illegible] his wife felt called to the missi[illegible] [illegible] by the Presbyterian Board of W[illegible] [illegible] /educational missionaries to M[illegible] [illegible] post in Tololoapan, Guerrero [illegible] completion of one year of theological trai[illegible] [illegible] minary and one year of language training in [illegible] Mr. Barnett's duties during that time were [illegible] to those of an agricultural extension agent in an [illegible] rable in size to the state of Florida

[illegible] Barnett attended Murray State University, Murray, [illegible] during his furlough year (1969-70), and began work [illegible] Master of Science degree in agriculture. He and his [illegible] returned to Mexico in 1970. The Presbyterian Church [illegible] ico voted at that time to request that all missionaries [illegible] removed from Mexico to allow complete autonomy. Mr [illegible] ett returned to Murray State University in 1972 and [illegible] leted work on his Master of Science degree in August, [illegible]

Mr Barnett began work toward a Ph.D. in Genetics in [illegible] the University of Florida. He taught the Genetics [illegible] (AY363) six quarters and wrote the manual which is [illegible] being used in that course. He taught the genetics [illegible] (AY362) during the fall of 1974.

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Associate Professor of Agronomy

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James Soule
Professor of Fruit Crops

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Professor of Agronomy

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Ralph C. Robbins
Associate Professor of Food Science

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